DISPOSITION OF PEER REVIEW COMMENTS FOR

Interaction Profile for Atrazine, Deethylatrazine, Diazinon, Nitrate, and Simazine

Prepared by:

Syracuse Research Corporation
Under Contract No. 205-97-0622

Prepared for:

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

July 2003

Peer Reviewers for the second draft of this interaction profile were:

Dale Hattis, Ph. D.
The George Perkins Marsh Institute
Center for Toxicology, Environment, and Development
Clark University
Worcester, MA

Kannan Krishnan, Ph. D.
Department of Environmental and Occupational Health
Faculty of Medicine
University of Montreal, Montreal, QC, Canada

Sheldon Wagner, M.D.
Department of Environmental and Molecular Toxicology
Oregon State University
Corvallis, OR

ATSDR thanks these scientists for their review of the document. When the reviewer=s suggestions were followed, or when other revisions obviated the need to respond, no further response is provided herein. Revisions that may have obviated the need to respond included sections that were rewritten, moved, or deleted. Some of the editorial and format suggestions could not be followed without changing ATSDR established format. Additionally, several stylistic changes that were purely arbitrary were not incorporated. Other suggestions made by the reviewers that ATSDR decided not to follow are discussed below.

In the discussion that follows, "PR" refers to the appropriate page of the assembled peer review document, "P" indicates a page number in the Second Draft of the profile, and "&" indicates the paragraph number on that page.

Review Comments provided by Dr. Dale Hattis

PR7, Chapter 1: The reviewer thought that the rationale for inclusion of diazinon was not clear because a rational for suspecting an interaction with the other components of the mixture was not clearly articulated and the subsequent analysis did not reveal appreciable evidence for interaction.

RESPONSE: The rationale for selection of the mixture is presented on page 1, second paragraph continuing to top of page 2. The rationale is based on the co-occurrence of these chemicals in rural well water. The rationale for selection of the mixture did not take into account whether an interaction with the other components of the mixture was expected, but rather what mixture or mixtures rural populations who depend on domestic groundwater wells are exposed to. Nevertheless, there are data indicating that atrazine and simazine may potentiate the toxicity of diazinon, and this data is presented and discussed in the interaction profile, and serves as the basis for a weight-of-evidence determinations for interactions.

PR8,9,10,11,12,13: The majority of Dr. Hattis= comments (annotated with A#1@ on the peer review report) were concerned with the interaction between atrazine or simazine and nitrate/nitrite to form nitrosoatrazine or nitrososimazine. Dr. Hattis suggested that the following additional analyses be performed: a detailed analysis of the potency of nitrosamines in carcinogenesis bioassays as compared with potency in *in vitro* human cell clastogenessis assays, followed by a distributional analysis for the potency of nitrosoatrazine and nitrososimazine clastogenesis relative to other nitrosamines, so as to predict carcinogenic potency, a similar review of the relative potency of other nitrosamines in fetal and early postnatal periods relative to adults, and a quantitative distributional evaluation of the potential carcinogenic risks arising from exposure to those nitrosamines, taking into account the degree to which nitrate/nitrite, atrazine, and simazine in water and *in vivo* will give rise to increased exposure to nitrosoatrazine and nitrososimazine. Dr. Hattis further commented that he thought the BINWOE scores for atrazine and simazine with nitrate should be higher.

RESPONSE: Preussmann and Stewart (1984), in an extensive review of N-nitroso carcinogenicity, evaluated the data on genotoxicity and carcinogenicity for nitrosamines. Of the 232 nitrosamines that had been tested for carcinogenicity, 199 (86%) were positive. Of the nitrosamines that did not give positive results, many were not tested at the maximum tolerated dose and/or were not tested in more than one species; thus the negative results were not definitive, and the actual percentage of nitrosamines that are carcinogenic may be higher than 86%. Few data are available regarding genotoxicity in mammalian cells, so correlations between mammalian genotoxicity and carcinogenicity cannot be investigated (Lijinsky 2001; Preussmann and Stewart (1984). Although there are reports of transplacental carcinogenesis for some nitrosamines, these were short term high dose studies for only a few members of this class, with no comparison to adults, or comparison to the dams only, so the data do not support an extensive analysis of relative sensitivity. In the 1976 NCI Monograph 51, which reported transplacental studies with 10 nitrosamines in hamsters, the F1 hamsters were less sensitive than the P females (i.e., had lower incidences and longer latencies); this finding does not indicate a higher relative sensitivity in the young as compared with adults. Thus, the analyses suggested by Dr. Hattis are not feasible.

What has been done in response to these comments is to include a more extensive discussion of nitrosamine carcinogenesis in the interaction profile. In addition, the weight-of-evidence ratings have been revised to reflect more confidence in the potential for greater-than-additive impact of the interaction on carcinogenicity.

PR11, Pv, &3: Dr. Hattis did not agree that the guidance that two or more components of a mixture should exceed a hazard quotient of 0.1 before a significant interaction is suspected should be applied to

the interaction between nitrate/nitrite and the triazine herbicides because the resulting new chemicals (nitrosamines) have a different mode of action from the parent chemicals.

RESPONSE: The yields of these nitrosamines *in vivo* following gavage administration of the parent compounds were very low (e.g., 0.04% for nitrosoatrazine in mice. The yields *in vitro*, including in human gastric juice, were dependent on the initial doses, with low percentage yields at relatively low initial concentrations of parent compounds. Therefore, the HQ of 0.1 for the parent compounds is a useful screen even for nitrosamine formation.

PR11, P2, 2nd to last sentence of the second new paragraph: Dr. Hattis stated that if the observed LOAELs for some experiments are lower than the corresponding NOAELs then the LOAELs should become the basis for RfDs and MRLs, and the NOAELs form the less sensitive experiments become irrelevant.

RESPONSE: As explained in the sentence on page 2, the lowest LOAEL was a ***serious*** LOAEL. ATSDR did not derive an MRL based on this LOAEL because ATSDR does not base MRLs on serious LOAELs. The text has been revised to eliminate confusion and to clarify this point.

PR13, P52, &4: Dr. Hattis questioned why the authors of the interaction profile and reportedly EPA believe that no additional FQPA safety factor was needed. FQPA 10-fold safely factors are needed unless adequate evidence was available to show safety for the developing nervous system. Without providing references or links to information online, the reviewer stated that the FQPA science advisory board concluded that this mandate for a 10-fold safety factor was not satisfactorily overcome by EPA=s 2002 analysis of developmental effects for the organophosphate common-mechanism group.

RESPONSE: In 2002, EPA released a revised OP cumulative risk assessment which included a reconsideration of the FQPA Safety Factor as it applies to ***cumulative*** risk assessment of the organophosphorus pesticides. It is not clear whether that is the document referred to by Dr. Hattis. A search of the EPA website did not reveal any documentation by the FQPA science advisory board. The information presented in the interaction profile does not reflect any belief of the profile author or of ATSDR. It is simply a factual account of the derivation of the RfDs and PADs by EPA (2000). That derivation has not changed as per the OPP diazinon website. The wording of the sentence has been changed to clarify the derivation and to eliminate the word Aneeded.@

Review comments provided by Dr. Kannan Krishnan

PR17, entire document: Dr. Krishnan stated that the document, overall, is a very good example of how interaction profiles should be developed for mixtures of concernCon the basis of critical effects of components and relevant joint toxicity information. He concluded that the document should be useful to public health officials and general public concerned about the health risks of combine exposure to the components of this mixture.

RESPONSE: None required.

PR18, P 1-3: Dr. Krishnan stated the purpose and rationale are clearly presented, but felt that presentation of conclusions of some of the mixture studies (triazines plus nitrite react chemically to form nitrosamines) on page 3 may be confusing.

Response: The introductions to these interaction profiles not only state the purpose and rationale, but also provide an introduction to the expected health effects of the individual components and the endpoints of concern for the mixture. In order to provide a rationale for carcinogenic effects as an endpoint of concern when the individual components are not carcinogenic, ATSDR feels that the formation of nitrosamines, which generally are carcinogenic, needs to be mentioned in the introduction.

PR18, PR39-53, Chapter 2: Dr. Krishnan listed three additional studies for consideration in the profile:
a. NTP study on pesticide/fertilizer mixture including atrazine, simazine, and nitrate
b. Study of *in vivo* simazine nitrosation in rats by Dmitrenko et al. (1996)
c. Study of N-nitrosoatrazine carcinogenicity reported only as an abstract (Weisburger et al. 1990)
d. Another report of greater-than-additive toxicity of diazinon and atrazine (Anderson and Lydy 2002) which corroborates Belden and Lydy=s observations.

Response:
a. The mixture in this NTP study also contained 1,2-dibromo-3-chloropropane, 1,2-dichloropropane, ethylene dibromide, and aldicarb, as well as atrazine, simazine, and nitrate, and was tested only as a complete mixture. Therefore, useful conclusions regarding the joint toxic action of atrazine, simazine, and nitrate cannot be extracted from this study, and it has not been added to the profile.
b. The study by Dmitrenko et al. 1996 is already cited on pages 12, 19, and 28 of the interaction profile.
c. ATSDR is aware of the study of N-nitrosoatrazine carcinogenicity, and has listed it in the reference list for this interaction profile (page 32), but ATSDR generally does not cite studies that have been published only as abstracts, because of concerns regarding study quality. ATSDR attempted to obtain a full manuscript or the study data from the principal author, but the principal author declined to provide it.
d. The Anderson and Lydy (2002) paper has been added to the Interaction Profile.

PR19, P23: Table 6 should indicate that the assessments should take into account the N-nitroso compounds resulting from chemical-chemical interactions.

RESPONSE: A footnote has been added to the table to deal with this issue. There are no toxicity data for these compounds from which to derive any health guidance values, such as TTDs.

PR19, P16 and P24: The value in Table 7 for atrazine/deethylatrazine on diazinon should be 0.23 to agree with that on page 16 (Table 2). This comment is superceded by Dr. Krishnan=s recommendation (PR19) of a score of 0.71 (B) for toxicological significance rather than 0.32 (C).

RESPONSE: ATSDR agrees that the score for toxicological significance should be changed to 0.71 (B); the change has been made and the resulting total score (0.5) has been entered in Tables 2 and 7.

PR20, P18-19Atrazine and Nitrate, Simazine and Nitrate: The reviewer stated that the score for mechanistic understanding should be 1 because the understanding of the interaction (as a chemical reaction) is well understood. In addition, the modifying factor should be 1 since the formation of the nitrosamine has been demonstrated in vivo.

RESPONSE: Mechanistic understanding has to do with the mechanism as it relates to direction of interaction (greater than additive, less than additive, or additive) with regard to toxicity. Thus, because there was little information regarding the action of the resulting nitrosamine, mechanistic understanding was considered poor in the draft sent to the reviewer. Additional evaluation of the data for the nitrosamine chemical class, however, has now been added and supports a higher rating (B) for mechanistic understanding, and also the deletion of the modifying factor.

PR21, P42: The reviewer questioned why deethylatrazine and atrazine are assumed to have equivalent toxicity on the basis of mg/kg/day, and thought that equivalency conversions should best be done on the basis of moles.

RESPONSE: On the basis of extensive test data for these two compounds, EPA has concluded that they have equivalent toxicity and that the RfD and PAD (in mg/kg/day) are to be applied to atrazine together with its chlorinated metabolites, including deethylatrazine. ATSDR is simply recommending that this value be adopted as a provisional TTD for atrazine together with deethylatrazine until such time as the toxicological profile atrazine is finalized, at which time the TTD should be reevaluated in light of conclusions of that profile.

PR21, P47: The reviewer suggested that a molecular weight conversion would be appropriate in establishing the TTD for simazine from that of atrazine.

RESPONSE: The difference in molecular weight is small, and there is no strong evidence that simazine and atrazine toxicity correlate better with molecular weight than with mass. Such a conversion would lend an aura of precision to this derivation by analogy that is unwarranted. ATSDR is recommending this value as a provisional TTD only; it should be reassessed at such time as MRLs or updated RfDs become available for simazine.

Review Comments provided by Dr. Sheldon Wagner

PR29, P7: The review wondered whether a discussion of olfactory effects in salmon which he stated have been seen with chlorpyrifos (another organophosphorus pesticide) should be added.

RESPONSE: The paragraph in question is in the section on joint toxic action of atrazine and diazinon. It discusses the mechanism of action of diazinon=s neurological effects (i.e., acetylcholinesterase inhibition), and how this mechanism may relate to the effects seen in a joint toxic action study of atrazine and diazinon in midges, and to the potential relevance of the midge study to humans. The suggested information does not contribute to the discussion. No reference for this information was provided by the reviewer, and no pertinent reference for this information was found during searches of MEDLINE and TOXLINE.

PR29, P9: Dr. Wagner stated, regarding the study by Van Leeuwen et al. 1999), that it was his professional opinion that this was considered an important study and that it raised doubts about nitrosamines carcinogenicity in humans, regardless of the shortcomings.

RESPONSE: The study is one of many epidemiological that have investigated possible associations between nitrate and cancer or between atrazine and cancer; there is no clear weight of evidence for carcinogenicity of either chemical, as reviewed in the appendices to the interaction profile. A concluding sentence has been added to address the nitrosamine issue.

PR30, P11: The reviewer suggested that the sentence could be changed from AThis raises a concern that the formation of N-nitrosoatrazine through chemical interaction may be a greater-than-additive interaction in terms of genotoxic and proliferative effects@ to A This raises a concern about unresolved scientific issues about the formation of N-nitrosoatrazine...@

RESPONSE: The available data show that N-nitrosoatrazine is formed *in vivo* and that it is more genotoxic and mitogenic than atrazine. Therefore the concern stated in the interaction profile is supported by the data.

PR30, P15: Table 1 Atrazine/Deethylatrazine and Simazine: The reviewer felt that the species difference should decrease the rating for the toxicological significance of the effect from A to B.

RESPONSE: The experimental results in fish are supported by the demonstrated similar mechanism of action and similar reproductive effects of these chemicals in mammals. Taken together, the evidence is considered by ATSDR to be strong enough to support the rating of A. ATSDR agrees that the fish data alone would not warrant this rating.

PR 30, P21: The reviewer expressed a concern regarding the sentence AIf only one or if none of the mixture components has a hazard quotient of this magnitude [0.1], no further assessment of the joint toxic action is needed because additivity and/or interactions are unlikely to result in significant health hazard.@ He felt that if a health hazard is exceeded by one component, no further assessment is needed because a health hazard is already established, and that therefore the term Aunlikely@ seems inappropriate.
RESPONSE: The hazard quotient (HQ) of 0.1 does not indicate that a health hazard is exceeded for a single component; rather a HQ of >1 for a single component indicates there is a potential for health hazard and that further assessment may be needed. The term Aunlikely@ is appropriate, because the sentence is

stating that *additivity and/or interactions* are unlikely to result in significant heath hazard when only one or when none of the chemicals are present at exposure levels that exceed a HQ of 0.1.

PR30, P22: The reviewer questioned whether the types of data to be considered during additional evaluation (community-specific health outcome data and community health concerns) exist and if not felt that it should be stated that they do not exist.

RESPONSE: These types of data are collected by ATSDR during the course of the public health assessment process and are specific to the community impacted by the contaminants. The references at the end of the sentence include further information, which is outside the scope of the interaction profile.

PR30-31; P38, 40, 46 (in Appendixes A and B): Several of Dr. Wagner=s comments (annotated with A#2@ on the peer review report) asked whether levels of atrazine found in the environment (in the epidemiology studies) should be compared with Athe relative dose of exposure@ (not further explained in the comments) in the experimental animal studies, and that the levels of exposure in the animal studies in Appendixes A and B be stated.

RESPONSE: The epidemiology studies did not provide estimates of dose. A few provided estimates of water concentrations, but water usually was not the only medium of exposure. The text regarding animal studies is a review of a large number of animal studies, which had been conducted by placing the chemical in the lab chow, and for which dose was reported in the referenced toxicological profile only as mg/kg body weight/day. To insert doses for each study into the text is not practical, and would not further a comparison with the human studies which do not contain dose estimates. Doses are, however, provided for critical effects in the section on Health Guidelines in each appendix.

PR32, P58: The reviewer thought that it would be helpful to the reader, in addition to TTD doses of mg/kg/day for nitrate, if ATSDR would consider adding the levels in ppm because in the case of nitrates, regulations are based more on ppm in the water or foods, etc.

RESPONSE: The regulations for levels of nitrate in water, although they may be expressed as ppm (or mg/L), are based on the RfD in mg/kg body weight/day, as well consideration of the contribution to exposure from ingestion of other media (such as food), and other, non-health-related considerations. Oral MRLs and RfDs are always expressed as mg/kg body weight/day. Expressing oral MRLs, RfDs, and TTDs in mg/kg body weight/day allows for comparisons with ingested doses regardless of what media were ingested (food, water, soil) and with aggregate doses from ingestion of more than one contaminated medium.